Original Research

Body Weight and Prior Depletion Affect Plasma Ascorbate Levels Attained on Identical Vitamin C Intake: A Controlled-Diet Study

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Key words: ascorbic acid, vitamin C, nutrient requirements, obesity

Purpose: To evaluate the role of factors that may affect the level of plasma ascorbic acid (AA), including age, body weight, physical activity, minor illness and the impact of prior depletion and repletion.

Methods: After one month of stabilization on 60 mg vitamin C/day, subjects underwent two complete depletion-repletion cycles (one cycle=one month of vitamin C depletion with nine mg/day, followed by one month of repletion with 117 mg per day). Subjects (68 men, ages 30 to 59 years) did not smoke or drink alcohol during the study. All food was provided by the study.

Results: There was extreme individual variability in the plasma AA level achieved on an identical repletion dose: after four weeks at 117 mg/day of vitamin C, AA ranged from 26.8 μmol/L to 85.8 μmol/L. Body weight was inversely associated with plasma AA attained ($p<0.0001$). Regression analysis indicated that, compared to a 130-lb man, a 200-lb man reached 10 μmol/L lower AA after the first repletion and 18 μmol/L lower AA after the second repletion. One-third of the subjects did not reach a plasma plateau after the first repletion. Prior depletion and apparent repletion also had a major impact, and only 10% of subjects reached the same plasma AA on the second repletion as on the first repletion.

Conclusions: Plasma AA attained on a given dose depends on body weight (or dose per kg of body weight) and on whether or not any prior depletions had been repleted adequately. The results have implications for nutrition recommendations and research design.

INTRODUCTION

The National Cancer Institute, in collaboration with the U.S. Department of Agriculture, undertook an investigation of several aspects of ascorbic acid (AA) metabolism. These included the role of different food sources on plasma ascorbate (reported elsewhere [1]), and the effect of depletion and repletion on blood pressure [2]. In this paper we report data on factors that may affect plasma AA level, including dose, age, body weight, physical activity, stress and having undergone a prior depletion and repletion.

A number of investigators have examined vitamin C requirements by feeding subjects ascorbic acid-deficient diets and then feeding varying amounts of vitamin C. Observations in these studies pertained to the point at which deficiency symptoms were seen and the amount of vitamin C that caused the regression of those symptoms [3–9] or to the plasma plateau achieved on a given dose [10]. The largest of these studies included only 11 subjects. One study [8] included a partial second depletion-repletion cycle, and in that study the second repletion was for only one week, with nine subjects on two different doses. All of these studies found substantial variability in plasma level attained on any given dose, but had an insufficient number of subjects to investigate the extent or sources of individual variability in dose response. The only previous depletion-repletion study to include a substantial number of

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subjects was that of Blanchard *et al.* [11], who depleted 29 young and 29 elderly subjects and then repleted them with 500 mg/day.

The study reported here, with 68 subjects, provides more data on and quantification of sources of individual variability in plasma levels attained on nonpharmacologic doses of vitamin C. In addition, inclusion of a second complete depletion-repletion cycle provides the opportunity to examine whether conclusions about dose response that might be reached after a single depletion and repletion would be different after a second depletion-repletion cycle.

METHODS

Study Design. One month of stabilization on approximately 60 mg of vitamin C per day was followed by two complete depletion-repletion cycles of two months each (Table 1). During each one-month depletion, subjects consumed a controlled diet providing only nine mg/day of vitamin C. Each one-month repletion period provided approximately 117 mg/day of vitamin C. Subjects did not smoke, drink alcohol or take aspirin during the study, consumed only food and drink provided, and weight gain or loss was prevented by weekly adjustment of caloric intake. The study was approved by the Human Subjects Review Committees of the National Cancer Institute, the U.S. Department of Agriculture and the Georgetown University School of Medicine.

For repletion, subjects were randomized to one of three sources of vitamin C (an additional 108 mg of vitamin C, from either supplement, fruit or vegetables). Analyses of the effects of vitamin C source during repletion on plasma ascorbate levels are described elsewhere [1] and are not the focus of the present paper.

Rational for Dosage Amounts. The purpose of the study was to study the effects of physical factors and of different food sources on blood levels attained with *dietary* levels of vitamin C. Average vitamin C intake in the U.S. is typically in the 100 to 120 mg/day range. Since one of the factors we wanted to investigate was whether vitamin C obtained from a supplement produced the same blood level as the same amount obtained from fruit or vegetables, we first sought a commercial vitamin C supplement that provided 100 mg. After assaying several commercially available tablets of nominally 100 mg, we found one that was close to that amount and provided 108 mg (others were higher). This 108 mg, added to the depletion diet providing nine mg/day, resulted in a target daily amount of 117 mg/day for the Supplement Group. Diets in the Fruit and Vegetables Groups thus were also designed to provide a total daily dose of 117 mg/day during repletion.

Subjects. Participation was limited to healthy men 30 to 59 years of age, who had not smoked for at least six months. Subjects were recruited from the greater Beltsville, Maryland, area through advertisements in newspapers and local places of employment. Seventy-one volunteers enrolled, and 68 completed the entire study.

Diets. Subjects were fed a 14-day rotating menu composed of foods common in the U.S. diet, providing approximately 50% of energy from carbohydrate and 36% from fat [1]. Subjects were weighed weekly, and energy intake was adjusted to prevent weight change during the study. On weekdays, subjects consumed breakfasts and dinners at the study facility. Lunches and snacks were provided as bag lunches; weekend meals were provided, frozen and packed in coolers, and consisted of the same types of hot meals which were provided on weekdays. This type of controlled-diet study is identical to the approach used by the Dietary Approaches to Stop Hypertension (DASH) study sponsored by the National Heart, Lung and Blood Institute [12].

During the depletion periods, the menus were nutritionally adequate except for vitamin C. During repletion, subjects randomized to the Supplement Group ate the same depletion diet, with vitamin C added back in pill form. In the Vegetable or Fruit Groups, foods containing vitamin C were added to the depletion diet. The AA content of the repletion foods was analyzed twice weekly by HPLC, and the amount fed was adjusted to maintain a constant AA intake over the repletion periods and in all study groups.

Compliance. Subjects were required to consume all food provided and were closely monitored by study staff during

Table 1. Study Design, Duration and Dose for Each Study Period and Plasma AA Levels Attained at Each Time Point

Study Week	Vitamin C Dose	Plasma AA (μmol/L) (SD)
Week −3 (Start of stabilization)		45.3 (16.5)
	Weeks −3 to 1: 60 mg/day	
Week 1 (Start of 1st depletion)		39.8 (10.3)
	Weeks 1 to 5: 9 mg/day	
Week 5 (end of 1st depletion)		12.2 (3.1)
	Weeks 5 to 9: 117 mg/day	
Week 9 (end of 1st repletion)		59.1 (11.5)
	Weeks 9 to 13: 9 mg/day	
Week 13 (end of 2nd depletion)		13.4 (2.4)
	Weeks 13 to 17: 117 mg/day	
Week 17 (end of 2nd repletion)		48.6 (13.6)

meals; any uneaten lunch or weekend food was returned and weighed. Calculations at the end of the study indicated that uneaten food was negligible and had no effect on average vitamin C intake. A daily checklist report of protocol violations and a final, post-reimbursement anonymous questionnaire revealed that reported dietary omissions or commissions were minor and had no effect on average energy, vitamin C or other nutrient intake. These monitoring procedures indicated excellent compliance with the dietary regimen. These compliance measures and conclusions are similar to those reported by the DASH study [12], which used a comparable feeding study design.

Data Collection. Information was obtained at baseline about usual diet, physical activity, prior smoking history and current passive smoke exposure, stress and demographic characteristics. The relationship between plasma nutrient values at baseline and questionnaire nutrient estimates has been reported elsewhere [13,14]. During the controlled diet portion of the study, an event checklist was completed by the subjects daily, on which they indicated whether they had been sick, taken medications, engaged in physical exercise or experienced environmental or social stress. Subjects were weighed weekly. Body mass index (weight/height^2) was calculated, and fat mass and lean mass were measured by bioelectric impedance.

The factors obtained from the daily checklist (sickness, medication use, operation or dental work, exposure to environmental stress, experience of social/psychological stress, vigorous physical activity that day) were tallied and a score obtained for each study period. For example, a separate score was calculated for number of days of vigorous physical activity during each depletion and each repletion period. These derived variables were then examined as covariates in multiple regression analyses.

Plasma Analyses. Venous blood was obtained from fasting subjects, at approximately the same time of day, prior to breakfast. It was obtained at baseline, biweekly during the depletion periods and weekly during the repletion periods. Blood was collected in vacutainers containing EDTA, iced and centrifuged within 30 minutes. Plasma was stabilized with freshly prepared 10% meta-phosphoric acid, vortexed, stored at −70°C and analyzed within 10 days of collection. Plasma ascorbic acid concentration was determined spectrophotometrically using 2,4-dinitrophenylhydrazine [15], which has been shown to correlate highly with HPLC methods [16–19]. Ascorbic acid is presented here in mg/dL as well as in μmol/L, for comparison with earlier data.

Statistical Analyses. Statistical analyses were conducted using SAS and included *t* tests and multiple regression analyses, with significance at $p<0.05$. For some analyses, a measure of integrated tissue exposure to AA over a time period was estimated for each individual as the sum of the plasma concentrations at each week of repletion. We refer to this as "area under the plasma repletion curve."

RESULTS

Study participants consisted of 57 white subjects (including four Hispanics), eight African-American and three Asian subjects. Average age of subjects was 40.6 years (range 30 to 59 years) and average weight was 80.9 kg (178 lbs), range 59 kg to 101 kg. The values, determinants and correlates of plasma AA response in each study period are examined in detail below, for the controlled-diet phase of the study, Weeks 1 to 17.

Depletion on Nine mg/Day

Plasma AA fell from a mean of 39.8 μmol/L (0.70 mg/dL) at the beginning of depletion to a mean of 12.2 μmol/L (0.21 mg/dL) after one month on nine mg/day during the first depletion cycle and to 13.4 μmol/L (0.24 mg/dL) during the second cycle (Table 1). It was not our intention to reduce subjects to scorbutic levels, and no symptoms of scurvy were observed.

There was considerable variability in the AA level to which subjects fell on the depletion dose, with individual respondent values ranging from 13.6 to 29 μmol/L (0.24 to 0.51 mg/dL). The plasma AA value at the beginning of depletion was positively associated with the level reached on depletion ($r=0.52$). Excluding initial plasma AA level, total body weight was highly significant and a slightly stronger predictor of the extent of the fall in AA than was lean body mass. In multiple regression analysis, no other factors affected the extent of the fall in AA on depletion, including age, BMI, reported physical activity or use of vitamin supplements prior to the start of the study.

Repletion on 117 mg/Day

At the end of the Cycle 1 repletion, mean plasma AA reached 59.1 μmol/L (1.04 mg/dL); after the Cycle 2 repletion, mean plasma AA reached only 48.6 μmol/L (0.86 mg/dL). Both mean AA at the end of repletion and area under the repletion curve were significantly different between Cycle 1 and Cycle 2 as evaluated by paired *t* tests ($p<0.0001$) (data not shown). In Cycle 2 only 10% of subjects equaled or exceeded the plasma AA levels they reached on the first repletion.

The vitamin C dose per kg of body weight was strongly associated with extent of repletion as measured by total area under the repletion curve (AUC) (Fig. 1). This association ($r=0.66$) was stronger than that between dose per kg of lean body mass and AUC ($r=0.57$) or that between dose per kg of body weight and AA reached at the end of the first repletion ($r=0.42$) (data not shown).

To investigate factors associated with extent of repletion, individuals were ranked into quartiles (Q1=low, Q4=high) by total area under the repletion curve (AUC). Characteristics of the sample by quartile of AUC in Cycle 1 are shown in Table 2. Those subjects with the greatest repletion (Q4) were younger and considerably lighter than those with the least repletion (trend $p<0.0001$). Body weight was 71.0 kg (156 lbs) in the high-repleting group, compared with 88.3 kg (194 lbs) in the

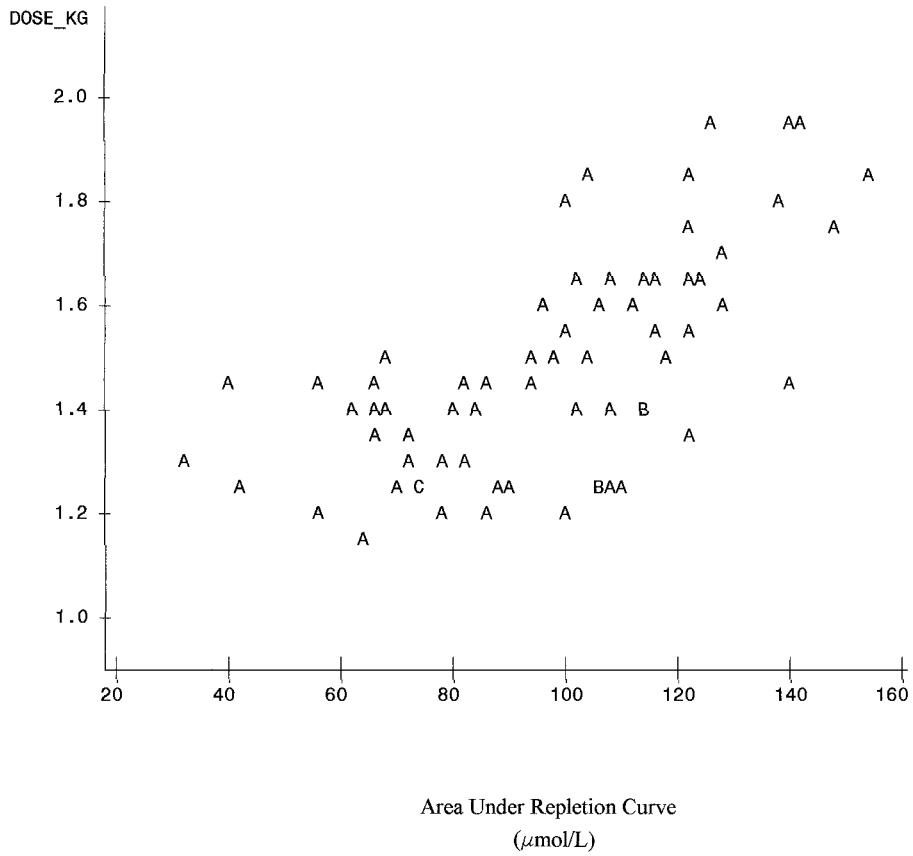

Fig. 1. Plot of relationship between vitamin C dose per kg of total body weight and area under the repletion curve, first repletion cycle, n=68 men, r=0.66. Association of dose per kg of lean body mass with AUC is lower, r=0.57.

Table 2. Characteristics of Subjects, Overall and by Quartile of Area under the Plasma Repletion Curve (AUC)[1], Depletion/Repletion Cycle 1

	All Subjects	Quartile of Area Under the Repletion Curve				Significance of trend (p)
		AUC Quartile 1 (Low)	AUC Quartile 2	AUC Quartile 3	AUC Quartile 4 (High)	
Number	68	17	17	17	17	
Mean AUC (μmol/L)	130.8	84.0	120.6	145.9	172.6	<0.0001
(SEM)	(4.4)	(5.2)	(2.6)	(1.3)	(4.0)	
Age (year)	40.6	42.5	43.9	38.4	37.6	0.02
(SEM)	(1.0)	(2.1)	(2.1)	(1.8)	(1.4)	
Weight (kg)	80.9	88.3	84.4	79.7	71.0	<0.0001
(SEM)	(1.3)	(1.7)	(2.5)	(2.0)	(2.5)	
Lean body mass (kg)	65.6	68.9	67.3	66.1	59.9	<0.0001
(SEM)	(0.8)	(1.0)	(1.7)	(1.5)	(1.6)	
Plasma AA at start of repletion (μmol/L)	12.2	10.7	11.4	12.8	14.0	<0.0004
(SEM)	(0.4)	(0.6)	(0.7)	(0.6)	(0.8)	

[1] Area under the repletion curve during the four repletion weeks of Cycle 1 was divided into quartiles.

low-repleting group. These same relationships between weight and plasma repletion were seen in the second repletion cycle as well (data not shown).

Factors affecting repletion were next examined in multiple regression analysis with area under the curve as the outcome variable. Plasma AA at the beginning of repletion (i.e., at the end of the depletion period) was a significant predictor in both cycles, as was intake per kg of body weight. There was no significant effect of age, over the age range in this study (30 to 59 years). The major treatment groups (Supplement, Fruit, Vegetable) were not significant factors (although some differences among subgroups were seen and are reported elsewhere

[1]). Other variables, including energy intake, exercise, sick days, stress, medications, passive smoke exposure (all potentially related to increased catabolism), uric acid at baseline and consumption of caffeinated beverages (potentially related to urine losses) were examined and did not contribute to the explanation of differences in plasma AA levels. The final regression models for the two repletion phases were as follows:

$$\text{Area}_1 = -61.1 + 3.3*\text{AA}_5 + 99.2*\text{dose}_{kg}, \quad R^2 = 0.45,$$

$$\text{Area}_2 = -168.5 + 7.6*\text{AA}_{13} + 95.7*\text{dose}_{kg}, \quad R^2 = 0.55,$$

where Area_1 and Area_2 are the areas under the plasma AA repletion curve for Cycles 1 and 2, respectively; AA_5 and AA_{13} are the plasma AA values at Weeks 5 and 13, the beginning of each repletion cycle; and dose_{kg} is the vitamin C intake per kg of body weight. In both cycles, intake per kg of body weight was significant at $p<0.0001$. When body weight is substituted for intake per kg of body weight in the above models, a negative coefficient but an essentially identical R^2 is achieved, as would be expected, since they are simply the same variable (body weight) multiplied by a constant (117 mg/day). In the context of a study such as this, in which vitamin C intake was the same among all subjects, body weight may be interpreted as an effect modifier of the relationship between intake and resulting plasma repletion.

Based on these regression models, estimated plasma levels that would be attained after various time intervals in persons with various body weights and depletion histories were calculated (Table 3). After two weeks of 117 mg vitamin C per day, the 59 kg (130-lb) subject will have achieved a plasma AA value of 60.8 μmol/L (1.07 mg/dL), while the 91 kg (200-lb) subject will have achieved only 36.4 μmol/L (0.64 mg/dL). Table 3 also indicates that the plasma AA value attainable at a given dose is not only a function of the dose and body weight (or dose per kg of body weight), but also a function of the pre-existing depletion experience and presumably, therefore, of tissue status. In the second cycle, subjects achieved a lower plasma AA and took approximately two weeks longer to reach the AA level that they had achieved in Cycle 1. For example, subjects in the 59 kg group reached 60.8 μmol/L after two weeks in the first repletion, and took four weeks to reach 59.1 μmol/L in the second repletion (Table 3).

Individual Variability in Response

Fig. 2a shows the responses to the various doses in this study, for each of the 68 men. An extraordinary variability is seen in plasma level achieved after one month on 117 mg vitamin C per day, in each of the two repletion cycles. Plasma AA ranged from 26.8 to 85.8 μmol/L at the end of the first repletion and from 16.9 to 75.6 μmol/L at the end of the second repletion.

Differences in body weight explain some of this variability (Figure 2b). At the end of the first repletion, mean plasma AA in the first and fourth body weight quartiles was 65.8 and 54.2 μmol/L, respectively ($p=0.0001$). Only subjects in the two lower body weight quartiles appear to be reaching a plateau after one month on 117 mg/day.

However, a great deal of individual variability in response to a given dose remains, even within body-weight groups. Figure 2c shows the variability within a single body-weight quartile. There was approximately a two-fold range in the plasma AA value achieved on repletion, *within a single-body weight quartile.* For example, in Body Weight Quartile 4, the

Table 3. Predicted Plasma AA (μmol/L) after Repletion with 117 mg/day AA for 1–4 Weeks, Assuming All Start at Same Depleted Level, by Body Weight[1]

	Plasma AA (μmol/L) Body Weight in kg (lbs)			
	59 kg (130 lbs)	68 kg (150 lbs)	82 kg (180 lbs)	91 kg (200 lbs)
Dose per kg body weight	1.98	1.72	1.43	1.29
Predicted plasma AA during repletion, after a single depletion				
Weeks on 117 mg/d				
0	11.4	11.4	11.4	11.4
1	26.7	24.4	20.5	17.6
2	60.8	53.4	43.2	36.4
3	68.2	63.6	56.8	52.3
4	66.5	63.6	59.1	56.2
Predicted plasma AA during repletion, after two depletions				
Weeks on 117 mg/d				
0	11.4	11.4	11.4	11.4
1	29.0	27.3	25.0	23.3
2	44.3	39.8	32.4	27.8
3	55.7	49.3	40.3	34.7
4	59.1	54.0	46.0	40.9

[1] Predictions based on coefficients in multiple regression analyses of study subjects.

value at the end of the first repletion ranged from 34.0 to 70.7 μmol/L, and the area under the repletion curve ranged from 72.8 to 162.0 μmol/L.

To explore the effect of such variability on results of studies with small sample sizes, we drew nine random samples of seven subjects each, and reexamined our data. These "studies" of seven subjects produced widely differing conclusions. For example, in one sample, subjects achieved a plasma AA of at least 37.5 μmol/L after one month on 117 mg/day, while in a different seven-person sample, subjects achieved a plasma AA of at least 68.7 μmol/L. The standard deviations in these nine samples ranged from 2.8 μmol/L to 17.0 μmol/L (data not shown).

DISCUSSION

This study represents the largest controlled-diet study of ascorbate depletion and repletion ever conducted. It is the first study to examine two full-length cycles of depletion and repletion, the first to include a substantial number of men in their 40s and 50s and the first to examine the effect of numerous behavioral and psychosocial factors reported by subjects on a daily basis. As such, it affords the opportunity to examine the role of a number of factors in influencing the plasma level attained on a fixed dose of ascorbic acid.

The level of plasma AA reached after one month of depletion is consistent with the results of other investigators [3,6,8,11,20]. The depletion results confirm those of Kallner *et al.* [21], that initial plasma level is the principal factor determining how far plasma levels will fall on depletion and that body weight is the only other influential factor.

The extreme variability observed in these data after repletion with an identical, fairly substantial dose (117 mg/day) can be seen in Figure 2, where levels ranged from 26.8 to 85.8 μmol/L after the first repletion and from 16.9 to 75.6 μmol/L after the second repletion. It is also seen in the large SDs after each repletion (Table 1), larger still after the second than after the first. This suggests that one month at 117 mg/day was insufficient to fill body pools and allow subjects to reach a plasma plateau. This variability was seen despite the fact that this was a relatively homogeneous study group: it did not include women, men in their 20s or over age 60, poor people, smokers, alcohol consumers or people with common chronic diseases such as high blood pressure, elevated cholesterol, diabetes, heart disease or the like.

The variability in plasma level attained on a given dose has also been observed by other investigators [4,10,21]. Levine *et al.* [10] found that the ultimate plasma plateau ranged from 14.9 to 58.8 μmol/L on an intake of 60 mg of vitamin C per day, and from 57.1 to 75.1 μmol/L on 200 mg/day, in seven young men. Unlike the present study, Levine *et al.* [10] continued each dose until a plasma plateau was achieved. They found that the time required to achieve a plateau also varied among the seven subjects.

The failure of 25% of our subjects to reach plasma levels of 40 μmol/L (the lower level of normal) in the second repletion of one month on 117 mg/day was unexpected. It suggests that repeated depletions may lead to an increasing impoverishment of body pool sizes and a widening gap in plasma AA levels between persons who have and have not experienced such repeated depletions. Previous research has not explored the effect of repeated depletions and the time and dose required to fully replete subjects.

This study quantifies the effect of body weight on the plasma level attained on a given dose. It indicates that on dietary intakes in this range, a heavy subject may attain a plasma AA level as much as 10 μmol/L lower than that of a light subject after a single depletion and as much as 18 μmol/L lower after a second depletion (Table 3). Others have observed the association with body weight, most often in the context of differences between men and women in plasma level [8,11,13,21–29]. Some authors have suggested a role for ovarian hormones or sex differences in renal absorption to explain observed gender differences in plasma concentration [30]. Others have suggested that gender differences, as well as the variability in plasma level in a single gender, may be due to differences in lean body mass or fat free mass [4,11,31,32]. The present data support the latter explanation: the gender effect, and variability in attained dose within a gender, may be substantially related to differences in body weight (a given dose in a smaller volume producing a higher concentration). In the present study we examined both total body weight and lean body mass and found similar results for both, perhaps because this was a single-gender study with relatively fewer differences in percent of body fat. Weight is used here because it is more commonly understood and measured. In addition, the multivariate analyses suggest that apparent age effects may be attributable to the weight gain that often accompanies aging, rather than to effects of aging *per se* (at least in the age range 30 to 59).

While it is clear from the present study that body weight is an important determinant of plasma level, and one that influences both the AA level attained on any given dose and the time required to reach that level, substantial unexplained sources of variability remained. Additional sources of variability in response may include the presence of several compartments in addition to plasma, varying degrees of tissue binding in those compartments [21] and genetic differences. A role for individual variations in emotional stress level [4] is not supported in the present data, nor are factors such as physical stress, minor illnesses or physical activity level. However, a role for such factors also cannot be ruled out; there may have been insufficient variability in those factors among persons in this sample, and a larger study and one designed specifically to

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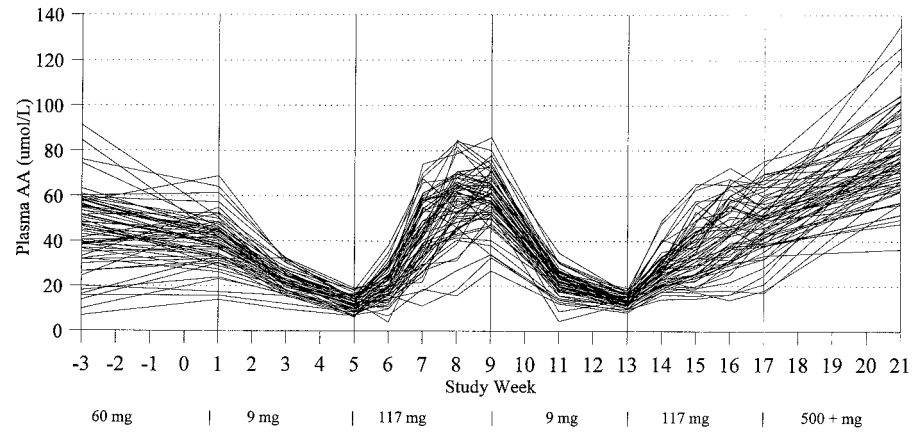
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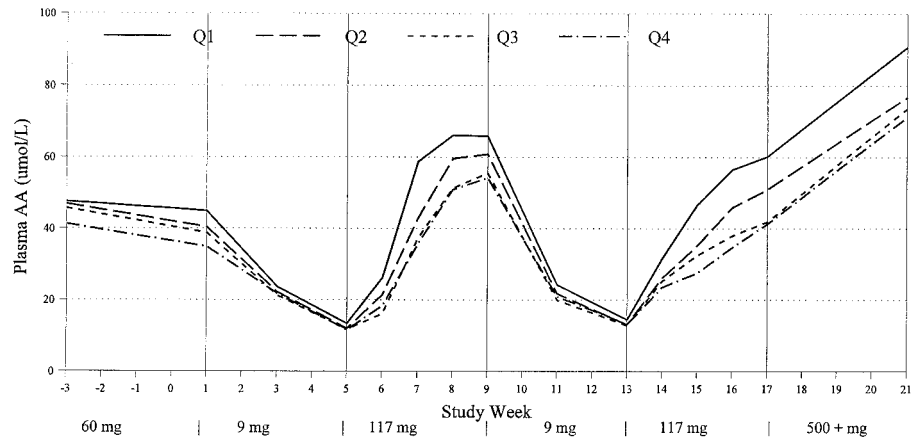
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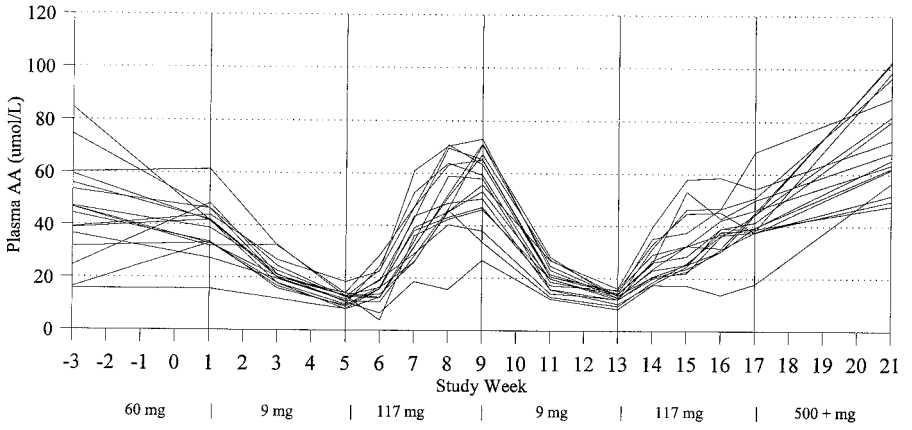
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answer such questions would be required. In addition, unmeasured environmental exposures could have contributed to differences in plasma level; however, we did obtain more information on a daily basis than had any previous research, including daily reports on passive smoke exposure and environmental stress, but were unable to identify factors that influenced the resulting blood levels.

In summary, these analyses have shown that the plasma AA that will be attained depends on several factors: the *daily intake* (compare the 9 mg, 60 mg and 117 mg levels in Table 1), *body weight or dose per kg of body weight* (Table 2 and Table 3), tissue or *body pool status*, reflected here in the range of levels reached on depletion and in the effect of previous depletion experience (compare AA levels after repletions one and two, in Table 1), *duration of repletion* (note incomplete repletion and absence of a plateau after one month, in Cycle 2 [Fig. 2b]) and *unknown individual factors* (Fig. 2a and 2c).

Several of these results may have implications for health policy and nutrition recommendations, clinical practice and research design. The role of body weight in partially explaining attained plasma level might be considered in making public health recommendations. In national survey data, approximately one-third of U.S. adults are overweight, and approximately one-third of men in this age range in the United States have plasma AA values below 40 μmol/L [22], sometimes considered the lower level of "normal" AA [33,34]. These data suggest that any recommended intake expressed in mg/day will result in lower blood AA levels in men than in women and lower blood AA levels in overweight than in normal-weight persons. The same would be true for recommendations regarding numbers of servings of fruits and vegetables. The extreme variability in response to a given dose of AA in repletion (Fig. 2a), over and above that explained by body weight, also supports the idea that it may be useful to express health-related recommendations in terms of desirable plasma concentrations, rather than in terms of milligrams of intake per day. The great advances in prevention of heart disease are due in part to campaigns to "know your (serum cholesterol) number" and guidelines that medical care practitioners can follow to identify *blood* levels in the desirable range.

At present there is no consensus on the importance of AA level in health promotion (although suggestive data exist [2,35–39]) and insufficient information on which to base health recommendations expressed in terms of plasma concentrations (although again, suggestive data exist [2]). However, such information can be amassed, if epidemiologic researchers, health surveys and clinicians routinely collect plasma AA data. In the face of variability in plasma response to intake, a reliance on dietary intake measures to evaluate nutrient-disease relationships can only lead to inadequate and perhaps incorrect interpretations.

The slower rate of repletion and lower plasma AA levels attained on the second repletion, after an apparently adequate first repletion, may also have public health implications. Although both repletions began with subjects at similar plasma levels, both provided an intake of 117 mg/day and both lasted for one month, the mean plasma AA was significantly lower on the second repletion than on the first ($p<0.0001$), and most subjects had not yet reached a plateau after one month. There were large differences between the first *vs.* the second repletion in the percent of subjects in who failed to reach 40 μmol/L (7% *vs.* 25%, respectively) or failed to reach 58 μmol/L (1.0 mg/dL) (37% *vs.* 75%, respectively). It may be that the first cycle depleted major body pools, which were not fully repleted after the first one-month repletion on 117 mg/day. In that case, repeated depletions which are not fully compensated may result in increasingly low circulating AA levels. Such repeated depletions may not be uncommon among the poor, among those with chronic illnesses or among the elderly who are subject to increasingly frequent illness or hospitalization. Numerous studies have found that hospitalized persons or those with chronic diseases have depleted plasma AA values [40–44].

These data also have implications for research design. First, it would appear that one month is insufficient for plasma levels to reach a plateau in all subjects, if the dose is in the dietary ranges, c. 100 to 150 mg/day. Second, studies should match treatment groups on body weight, stratify randomization within body weight groups or control for this factor. Finally, the great variation in both average levels and standard deviations seen with the random subsets of $n=7$ in this data set, and by others [21], emphasizes the difficulty in determining an accurate 2SD "margin of error" in recommendations. In his estimate of ascorbate requirements, Kallner [21] noted that, "Because of the low number of data points, the interindividual variation can hardly be calculated by correct statistical means and only rough estimations are possible;" his later study on smokers found that "a larger scattering was observed than in nonsmokers" [45]. Although this might have been due to differences in smoking habits, as theorized by Kallner, it may also have been due to variations resulting from small samples. The success of studies like this one, Blanchard *et al.* [11], and the DASH study [12]

Fig. 2. 2a. Plasma AA of each of the 68 study participants, over the course of the study.

2b. Mean plasma AA over the course of the study, within quartiles of body weight. Q1 (quartile 1) comprised persons in the lightest body weight quartile, range 59.3–71.3 kg; Q2, range 72.2–81.4 kg; Q3, range 81.5–90.9 kg; Q4 comprised persons in the heaviest quartile of body weight, range 91.5–100.8 kg. Mean area under the curve was different between the first and fourth body weight quartiles ($p<0.0001$), first and third ($p<0.0001$) and first and second body weight quartiles ($p<0.006$).

2c. Even within a single quartile of body weight, however, individual variability remains. Plasma AA of the 17 study participants in body weight quartile 3, over the course of the study.

demonstrate the possibility of conducting large-scale controlled-diet studies on free-living subjects. While exquisitely-controlled metabolic-unit studies on very small numbers will always have an important role in nutrition research, it would seem that larger samples may be needed to fully appreciate the complexities of human responses to this and other nutrients.

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REFERENCES

1. Mangels AR, Block G, Frey CM, Patterson BH, Taylor PR, Norkus E, and Levander OA: The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. J Nutr 123:1054–1061, 1993.
2. Block G, Mangels AR, Norkus EP, Patterson BH, Levander OA, Taylor PR: Ascorbic acid status influences diastolic blood pressure. Submitted, 1999.
3. Baker EM, Hodges RE, Hood J, Sauberlich HE, March SC, Canham JE: Metabolism of 14C- and 3H-labeled L-ascorbic acid in human scurvy. Am J Clin Nutr 24:444–454, 1971.
4. Baker EM, Hodges RE, Hood J, Sauberlich HE, March SC: Metabolism of ascorbic-1-14C acid in experimental human scurvy. Am J Clin Nutr 22:549–558, 1969.
5. Hodges RE, Baker EM, Hood J, Sauberlich HE, March SC: Experimental scurvy in man. Am J Clin Nutr 22:535–548, 1969.
6. Hodges RE, Hood J, Canham JE, Sauberlich HE, Baker EM: Clinical manifestations of ascorbic acid deficiency in man. Am J Clin Nutr 24:432–443, 1971.
7. Jacob RA, Pianalto FS, Agee RE: Cellular ascorbate depletion in healthy men. J Nutr 122:1111–1118, 1992.
8. Jacob RA, Skala JH, Omaye ST: Biochemical indices of human vitamin C status. Am J Clin Nutr 46:818–826, 1987.
9. Sauberlich HE, Kretsch MJ, Taylor PC, Johnson HL, Skala JH: Ascorbic acid and erythorbic acid metabolism in nonpregnant women. Am J Clin Nutr 50:1039–1049, 1989.
10. Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, Cantilena LR: Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci USA 93:3704–3709, 1996.
11. Blanchard J: Depletion and repletion kinetics of vitamin C in humans. J Nutr 121:170–176, 1991.
12. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin P-H, Karanja N: A clinical trial of the effects of dietary patterns on blood pressure. N Engl J Med 336:1117–1124, 1997.
13. Sinha R, Block G, Taylor PR: Determinants of plasma ascorbic acid in a healthy male population. Cancer Epidemiol Biomark Prev 1:297–302, 1992.
14. Sinha R, Patterson BH, Mangels AR, Levander OA, Taylor PR, Block G: Determinants of plasma vitamin E in healthy males. Cancer Epidemiol Biomark Prev 2:473–479, 1993.
15. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Clinical Chemistry Division: "Laboratory Procedures used by the Clinical Chemistry Division, for the Second National Health and Nutrition Examination Survey (NHANES II) 1976–1980. IV-Analytical Methods, Vitamin C." Atlanta: USDHHS, 1979.
16. Schaus EE, Kutnink MA, O'Connor DK, Omaye ST: A comparison of leukocyte ascorbate levels measured by the 2,4-dinitrophenylhydrazine method with high-performance liquid chromatography using electrochemical detection. Biochem Med Metab Biol 36:369–376, 1986.
17. Bates CJ, Bailey A, Vandenberg H, Vanschaik E, Coudray C, Favier A, Farre R, Frigola A, Heseker H, Maiani G, Ferroluzzi A, Pietrzik K, Thurnham DI: Plasma vitamin-C assays—A European experience. Int J Vit Nutr Res 64:283–287, 1994.
18. Tessier F, Birlouez-Aragon I, Tjani C, Guilland J-C: Validation of a micromethod for determining oxidized and reduced vitamin C in plasma by HPLC-fluorescence. Int J Vit Nutr Res 66:166–170, 1996.
19. Otles S: Comparative determination of ascorbic acid in bass (Morone lebrax) liver by HPLC and DNPH methods. Int J Food Sci Nutr 46:229–232, 1995.
20. Omaye ST, Skala JH, Jacob RA: Plasma ascorbic acid in adult males: effects of depletion and supplementation. Am J Clin Nutr 44:257–264, 1986.
21. Kallner A, Hartmann D, Hornig D: Steady-state turnover and body pool of ascorbic acid in man. Am J Clin Nutr 32:530–539, 1979.
22. Fulwood R, Johnson CL, Bryner JD: Hematological and nutritional biochemistry reference data for persons 6 months–74 years of age: United States, 1976–80. Vital and Health Statistics. Series 11-No. 232. DHHS Pub. No. (PHS) 83-1682. Washington, DC: Public Health Service, 1982.
23. Dodds ML: Sex as a factor in blood levels of ascorbic acid. J Am Diet Assoc 34:32, 1969.
24. Sauberlich HE, Skala JH, Dowdy RP: Laboratory tests for the assessment of nutritional status. CRC Crit Rev Clin Lab Sci 4:215–340, 1973.
25. Brook M, Grimshaw JJ: Vitamin C concentration of plasma and leukocytes as related to smoking habit, age, and sex of humans. Am J Clin Nutr 21:1254–1258, 1968.
26. Woodhill JM: Australian dietary surveys with special reference to vitamins. Int J Vit Nutr Res 40:520, 1970.
27. Loh HS, Wilson WM: Relationship of human ascorbic acid metabolism to ovulation. Lancet 1:110, 1971.
28. Sauberlich HE: Vitamin C status: methods and findings. Ann NY Acad Sci 258:438–450, 1975.
29. Heseker H, Schneider R: Requirement and supply of vitamin C, E and β-carotene for elderly men and women. Eur J Clin Nutr 48:118–127, 1994.
30. Garry PJ, VanderJagt DJ, Hunt WC: Ascorbic acid intakes and

plasma levels in healthy elderly. Ann N Y Acad Sci 498:90–99, 1987.
31. Jacob RA: Classic human vitamin C depletion experiments: Homeostasis and requirement for vitamin C. Nutr 9:74–86, 1993.
32. Baker EM, Sauberlich HE, Wolfskill SJ, Wallace WT, Dean EE: Tracer studies of vitamin C utilization in men: Metabolism of D-glucuronolactone-6-C14, D-glucuronic-6-C14 acid and L-ascorbic-1-C14 acid. Proc Soc Exp Biol Med 109:737–741, 1962.
33. "Current Medical Diagnosis & Treatment." Norwalk, CT: Appleton & Lange, 1992.
34. American Dietetic Association: "Handbook of Clinical Dietetics." New Haven: Yale University Press, 1981.
35. Simon JA: Vitamin C and cardiovascular disease: A review. Am J Clin Nutr 11:107–125, 1992.
36. Frei B, England L, Ames BN: Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 86:6377–6381, 1989.
37. Jacques PF, Hartz SC, Chylack LT, McGandy RB, Sadowski JA: Nutritional status in persons with and without senile cataract: Blood vitamin and mineral levels. Am J Clin Nutr 48:152–158, 1988.
38. Barer D, Leibowitz R, Ebrahim S, Pengally D, Neale R: Vitamin C status and other nutritional indices in patients with stroke and other acute illnesses: A case-control study. J Clin Epidemiol 42: 625–631, 1989.
39. Bates CJ, Walmsley CM, Prentice A, Finch S: Does vitamin C reduce blood pressure? Results of a large study of people aged 65 or older. J Hypertens 16:925–932, 1998.
40. Bodansky O, Wroblewski F, Markardt B: Concentrations of ascorbic acid in plasma and white cells of patients with cancer and noncancerous chronic disease. Cancer Res 11:238–238, 1951.
41. Schorah CJ, Morgan DB, Hullin RP: Plasma vitamin C concentrations in patients in a psychiatric hospital. Hum Nutr Clin Nutr 37:447–452, 1983.
42. LeGardeur BY, Lopez-SA, Johnson WD: A case-control study of serum vitamins A, E, and C in lung cancer patients. Nutr Cancer 14:133–140, 1990.
43. Marcus SL, Dutcher JP, Paietta E, Ciobanu N, Strauman J, Wiernik PH, Hutner SH, Frank O, Baker H: Severe hypovitaminosis C occurring as the result of adoptive immunotherapy with high-dose Interleukin 2 and lymphokine-activated killer cells. Cancer Res 47:4028–4212, 1987.
44. Akita S, Kawahara M, Takeshita T, Morio M, Fujii K: Effect of general anesthesia on plasma ascorbic acid level. Hiroshima J Med Sci 36:69–73, 1991.
45. Kallner AB, Hartmann D, Hornig DH: On the requirements of ascorbic acid in man: Steady-state turnover and body pool in smokers. Am J Clin Nutr 34:1347–1355, 1981.

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